



## Chromosomal imbalance report

A microduplication of 5p15.33 reveals *CLPTM1L* as a candidate gene for cleft lip and palate

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## ABSTRACT

We report a 10-year-old boy with syndromic cleft lip and palate (CLP) and neuro-psychomotor developmental delay. Oligoarray comparative genomic hybridization (aCGH) detected an approximately 300 kb interstitial microduplication at 5p15.33 encompassing 5 protein-coding genes, including *TERT* and *CLPTM1L*, and two microRNA genes. Our findings suggest that the duplicated segment predisposes for cleft lip with or without cleft palate (CL/P), or any of the other phenotypic features presented by the patient. A gene coding a similar protein (CLPMT1) has been implicated in CLP etiology both through linkage studies and by a translocation disrupting the gene, indicating the possible involvement of *CLPTM1L* with CL/P. This is the first report of a possible connection between *CLPTM1L* and CLP.

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## 1. Clinical description

The patient was a 10-year-old boy, the first child of healthy, non-consanguineous parents. Nuchal translucency and CLP were observed by fetal ultrasonography. He was born at term by caesarean section due to an increased amniotic fluid volume. Birth parameters were: weight 3570 g (50th centile); length 51 cm (60th centile). He had neuro-psychomotor developmental delay and was not able to sit stably until 12 months. He started walking independently at 26 months. Language started developing from 36 months onwards. At school age he was diagnosed with hyperactivity and attention deficit disorder. He exhibited downslanting palpebral fissures, low-set ears, prominent and wide nasal bridge, and CLP, which were surgically corrected when he was 3 years old; in addition he presented mild brachydactyly, atrio-ventricular septal defect (closed spontaneously), cryptorchid testes and micropenis.

## 2. Methods

Routine G-banding chromosome analysis from the patient were performed on metaphase preparations from peripheral blood lymphocytes using standard techniques at a resolution of 300–400 bands (low resolution) and showed an apparently normal (46,XY) karyotype. MLPA analysis was carried out to test using the 964B, 070 and 036 kits for subtelomeric regions (MRC-Holland, Amsterdam, Netherlands) and the results were normal.

DNA copy number was investigated by whole-genome array-CGH (44K, Agilent Technologies, Santa Clara, CA) performed according to the recommendations of the manufacturer. Data were processed with Feature Extraction software and subsequently analyzed with the Genomic Workbench software (both from Agilent Technologies). Gains and losses of genomic sequences were called using an aberration detection statistical algorithm ADM-2, with a sensitivity threshold of 6.7. Detected copy number variations (CNVs) were compared to data from oligoarray studies documented in the Database of Genomic Variants [DGV; <http://projects.tcag.ca/variation/>]. All variants present in at least four different studies in DGV were considered as normal population variants (common), and subsequently disregarded. Chromosome regions not previously reported as CNVs were singled out for further investigations, which included confirmatory studies by real-time quantitative PCR (qPCR) using the TaqMan® Copy

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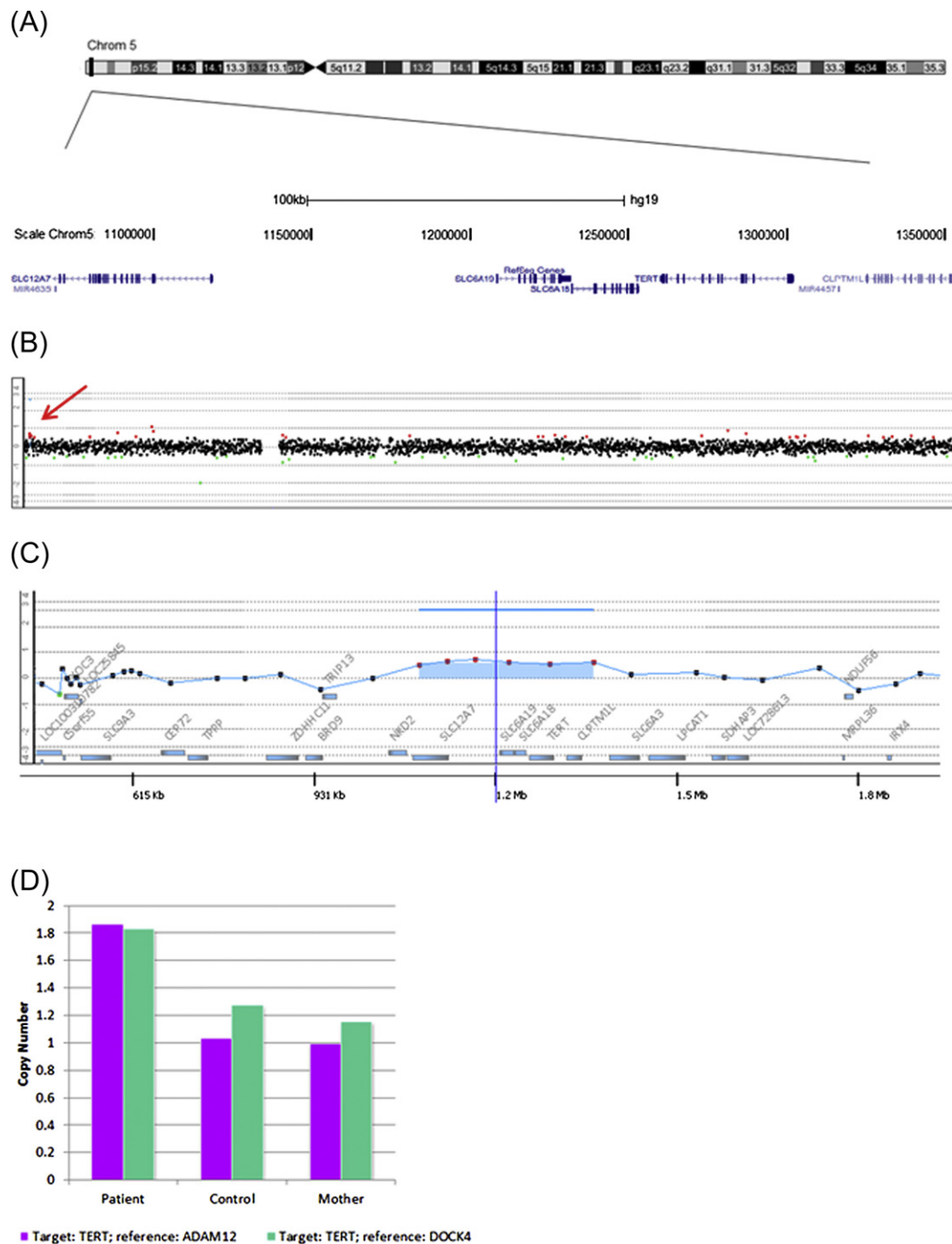
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Number Assays system (Applied Biosystems). For this, both patient's and mother's DNA were used in addition to a DNA sample from a control individual. We used the *TERT* copy number assay as target and compared the qPCR values with the *ADAM12* and *DOCK4* genes (normalization or reference genes). All samples were run in triplicate and the data analyzed in Microsoft Office Excel using an Excel script to compute the comparative  $\Delta\Delta C_t$  cycle threshold (Applied Biosystems), which assumes that the calibrator DNA has two copies of the control genes.

### 3. Genomic rearrangement

Array-CGH analysis revealed a microduplication at 5p15.33 (chr5:1,062,209–1,364,929; GRCh37/hg19) of approximately 300 kb (Fig. 1C) encompassing 5 Refseq genes [NCBI Reference Sequence; <http://www.ncbi.nlm.nih.gov/>], including *TERT*, *CLPTM1L* and three *SLC* family members (Fig. 1A, B, C). The alteration description with breakpoints according to ISCN (2013) is: arr 5p15.33(981,594x2, 1,062,209–1,364,929x3,1,429,655x2). The duplication was



**Fig. 1.** A. Ideogram of chromosome 5 showing the position of the duplication on the short arm (bar). In detail, the RefSeq genes located in the duplicated area. B. aCGH profile of the chromosome 5 of the patient. The red arrow points to the duplication site. C. High resolution view of the 300 kb duplicated segment at 5p15.33. D. Real-time quantitative PCR data. *TERT* gene copy number (three copies) was compared with those from *ADAM12* and *DOCK4* (two copies), confirming the duplication in the patient.

confirmed by quantitative real-time PCR. The qPCR experiment showed that the patient carried three copies of the *TERT* gene, while his mother and the control carried only two copies (Fig. 1D). Whether the duplication was *de novo* or inherited could not be definitely established since the father of the patient was unavailable for investigation.

#### 4. Discussion

Array-CGH detects genomic imbalances in 15–20% of patients with multiple congenital abnormalities and/or intellectual disability despite a prior normal karyotype, indicating the common involvement of microdeletions or duplications in many congenital diseases. The detection of CNVs has led to significant advances in identifying novel loci that contribute to many birth defects, including syndromic CLP, particularly where the clinical description does not closely fit that of well-known syndromes.

CLP is genetically heterogeneous and multifactorial, with several loci contributing to the phenotype. We report a patient exhibiting CLP, developmental delay, hyperactivity and minor dysmorphisms in conjunction with a submicroscopic 5p15.33 duplication. Syndromic forms of CLP account for 5%–7% of all cases and can result from interaction of multiple monogenic diseases, chromosomal rearrangements, and/or the effect of random environmental factors. This is the smallest duplication reported in this chromosome region area and also the first to associate duplication of 5p15.33 with syndromic CLP. The duplicated region includes 5 protein-coding genes: *CLPTM1L*; *SLC12A7*; *SLC6A18*; *SLC6A19*; *TERT*, and two microRNA genes: *MIR-4457* and *MIR-4635*. Little is understood about the functions of the Solute Carrier family genes (*SLC12A7*, *SLC6A18*, *SLC6A19*); however, several of these genes have been associated previously with neurological diseases, and it is plausible that duplication of these genes could have contributed to the developmental delay of this patient. Furthermore, it has already been observed that children with oral clefts may present also increased inattention/hyperactivity risks at older ages.

There is no overlap between the already known candidate genes for syndromic CLP and the genes in the detected duplicated region. The *CLPTM1L* (*CLPTM1*-like; OMIM 612585) gene – also known as *CRR9* (Cisplatin resistance-related protein 9) – encodes a protein that shares a 35% identity with *CLPTM1* (Cleft lip and palate-associated transmembrane protein-1), a protein encoded by a gene located at 19q13.3 whose disruption by a balanced translocation was reported in all members affected by CLP in a three-generation family [1] (reviewed in OMIM 604783). The same 19q13 region has been implicated also in non-syndromic cleft lip with or without cleft palate (NSCLP) through linkage and transmission disequilibrium studies [2], indicating a possible association between *CLPTM1* and NSCLP, although a 0.8 Mb deletion at 19q13.3 not encompassing *CLPTM1* has been reported, suggesting the presence of more than one CLP gene in the region [3]. Although *CLPTM1* and *CLPTM1L* share a common domain (InterPro database; <http://www.ebi.ac.uk/interpro/>), this is the first time that *CLPTM1L* has been associated with CLP. Due to the similarity that the *CLPTM1L* protein domain shares with *CLPTM1*, our findings raise a question on the role of the *CLPTM1* gene family in the cause of CLP. Yamamoto et al. [4] observed that overexpression of the *CLPTM1L* gene in human ovarian tumor cell lines induces *in vitro* apoptosis. In fact, *CLPTM1L* has been previously reported in the context of antidrug resistance, in particular in cisplatin-induced apoptosis. Accordingly, a duplication of the entire gene is likely to lead to increased gene expression, thus triggering overexpression of apoptotic pathways, which may contribute to phenotypical abnormalities, in particular CLP.

In addition to CLP, our patient had a congenital heart defect. *TERT* (Telomerase Reverse Transcriptase; OMIM 187270) gene alterations are related to several diseases, including susceptibility to Coronary Artery Disease, and duplication of this gene could possibly be related to the clinical manifestation of the patient. In fact, two other duplications with phenotypic description in DECIPHER and overlapping *TERT* gene show heart alterations. The combination of CLP, intellectual disability and heart defects is also observed in the 22q11 Deletion Syndrome (DiGeorge/Velocardiofacial Syndromes).

Little is known about the two microRNA (miRNA) genes that map within this duplication, but each of them has approximately 300 predicted targets [<http://mirdb.org/miRDB/>], including *DROSHA*, a ribonuclease III involved in miRNA processing, whose cofactor is *DGCR8* (DiGeorge Syndrome Critical Region Gene 8); the patient's phenotype, including intellectual disability, may be partially modulated by silencing of some of these predicted targets.

Partial or full trisomies of the short arm of chromosome 5 are rare, although a number of cases have been described [review in reference [5]; DECIPHER patient #4119]. The reported alterations overlapping 5p13.55 are mostly deletions and generally much larger than the alteration presented here. This is the first description of a clinical phenotype involving CLP being attributed to duplication of this small region; on the other hand, there is no report of involvement of CLP among the patients carrying large duplications encompassing the alteration in our patient, showing that if *CLPTM1L* indeed plays a role in this phenotype, it possibly shows a low penetrance which only manifests when a few genes are involved in the duplication.

Although we have discussed the role of individual genes in each phenotypic feature, the whole phenotype of the patient results from the duplication of the full chromosome segment. Documentation of additional cases is important to determine the role of the duplicated genes, isolated or in combination, in the phenotypic features of the patient. In particular, investigating copy number alterations of the genes in this region in a cohort of CLP patients with intellectual disability and hyperactivity should help establish a better phenotype–genotype correlation.

#### Conflicts of interest

The authors declare no conflict of interest.

#### Acknowledgments

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